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Tissue Plasminogen Activator for the Treatment of Adults With CriticalCOVID-19: A Pilot Randomized Clinical Trial

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Highlights

1. Widespread microthrombosis is considered as an etiology for impaired oxygenation in patients with COVID-19 complicating with ARDS.
2. Systemic thrombolytic versus prophylactic- and therapeutic-dose anticoagulation were compared in present pilot randomized clinical trial.
3. Systematic thrombolytic therapy did not improve oxygenation in critically ill patients with COVID-19.

Keywords: Coronavirus disease 2019 (COVID-19); acute respiratory distress syndrome (ARDS); Tissue plasminogen activator (tPA); Fibrinolytic therapy, Anticoagulation

LETTER TO THE EDITORS-IN-CHIEF

Microvascular and macrovascular thromboses are among the chief manifestations of COVID-19 (1) which play an important role in worsening respiratory status and acute respiratory distress syndrome (ARDS). The observed profound shunt fraction in patients with COVID-19 results into hypoxemia despite relatively preserved lung compliance and pressures (2). Reported endothelial injury and the widespread microthrombosis in postmortem studies (3), have been numbered as potential etiologies of the mentioned shunt fraction. Thus, such patients may benefit from more aggressive antithrombotic treatment upon hospitalization. Since the value of the therapeutic-dose anticoagulation have been questioned in the recent trials (1, 4), other antithrombitic regimens are currently under investigation (5). Recent series of critically ill patients showed at least a transient improvement in oxygenation following tissue plasminogen activator (t-PA) infusion (2) . Nonetheless, it is still uncertain to what extent the use of thrombolytics can improve oxygenation and survival. Moreover, the risk of bleeding in these patients necessitates that the decision as to whether or not to administer t-PA be made individually, which renders the conduct of large trials difficult.

In this open-label 3-arm pilot randomized clinical trial a total of 46 patients were screened for eligibility between September 2020 and April 2021, in 2 large tertiary academic centers in Tabriz and Zanjan, Iran (Supplementary Figure 1). Adult patients with positive SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR) assay within 5 days of the index hospitalization without known diagnosis of thrombosis, who were admitted to the ICU with PaO_2/FiO_2 (P/F) ratios <100 and D-dimer levels >3000 ng/mL were assessed for eligibility (Supplementary Table 1). The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1399.127) and accepted by the

otherenrolling center. Patients or their healthcare proxies provided informed consent for participation. Eligible patients were randomly assigned in a 1:1:1 ratio to one of the following 3 regimens: rtPA (to receive 25mg over 2 hours then 25mg for the next 22 hours of rtPA infusion, immediately followed by Unfractionated heparin (UFH) with a goal of activated partial thromboplastin time (aPTT) of 50-70 seconds), standard-dose prophylactic anticoagulation (to receive UFH at a loading dose of 80 IU/kg, followed by a continuous infusion of 18 mg/kg until aPTT of 50-70 seconds), and full-intensity prophylactic anticoagulation (TDA) (to receive 5000 IU of subcutaneous UFH every 8 hours).The dosage of thrombolytic regimen were based on limited available evidence on the role of thrombolytic therapy in COVID-19 era (2) along with previous experience on half-dose thrombolytic in pulmonary emboli (6). Considering the potential increased risk of bleeding in the concomitant thrombolytic and anticoagulation therapy (7), no parenteral anticoagulation was administered during the rtPA infusion and the assigned anticoagulation therapy in all patients was continued until hospital discharge. The primary outcome was the P/F ratio improvement during the first 48-hours of enrollment. Additional study outcomes included 30-day all-cause mortality, SOFA score improvement during the first 48-hours after enrollment, the rate of discharge from the ICU, the ICU length of stay, the hospital length of stay andacute venous or arterial thrombotic events. The main safety outcome was major bleeding according to the classification of the International Society of Thrombosis and Haemostasis in nonsurgical patients and severe thrombocytopenia (platelet count <20 × 103/μL). The study population was followed up for 30 days after enrollment. After discharge from the hospital, the patients were followed up weekly by phone interview.

Baseline characteristics of the 15 studied patients including 4 women (26.7%), at a median age of 60 (50 to 60) years are shown in Table 1. Time from symptom onset and time

from hospitalization duration until enrollment were 5 (4 to 6) days and 7 (5 to 9) days, respectively (Table 2).In patients in the thrombolytic arm, themedian percentage of the P/F ratio improvedduring the first 48-hour post-enrollment by7.1 (-19.5-8.4)%.The median difference of the SOFA score aggravatedwithin 48 hours of enrollment by1 (0 to 2) in this group. The ICU length of stay was 7 (6 to 10) days and no patients were discharge alive from the ICU. In the patients assigned to therapeutic-dose anticoagulation during the first 48 hours, the median percentage of the P/F ratio decreased by-7.6 (-14.5-68.9). The median difference of the SOFA score within 48 hours of enrollment was 0 (-1 to 0) in this group. The median ICU length of stay was 8 (4 to 19) days and three (60.0%) patients were discharge alive from the ICU. For patients received standard-dose anticoagulation, the median percentage of the P/F ratio changes was aggravated by -15.8 (-28.0-24.1)% within 48 hours of enrollment.The median difference of the SOFA score within 48 hours of enrollment was 1 (0 to 1). Only 1 (20%) patient of this group was discharged alive from the ICU and the median ICU length of stay was 11 (8 to 12) days.Overall, four of the 15 randomized patients were discharged alive from the ICU. All these patients had an uneventful hospitalization course. Four patients underwent computed tomography pulmonary angiography and 4 patients had lower extremity venous ultrasound studies. No acute arterial or venous thrombotic events were identified among study population.

Major bleeding and acute arterial or venous thrombotic events did not occur in any of our patients. Severe thrombocytopenia (platelet count: 16900) was reported in only 1 patient (20%) in the therapeutic-dose anticoagulation group on day 4.A median P/F ratio <100 and a median SOFA score >5 at baseline in all 3 study groups indicated the severity of COVID-19 involvement among our patients. The markedly increased baseline D-dimer (ng/mL) in our patients (median [IQR]: 10000 [7337 to 15000]) might be an indicator of possible poorer

outcomes (deceased cases: 11 [73.3%]). All our patients had acute respiratory support with noninvasive or invasive positive pressure ventilation at the time of randomization (5 [33.3%] and 10 [66.6%], respectively).

In the present pilot randomized clinical trial, none of the patients in the thrombolytic therapy arm developed major bleeding, however, the magnitude of improvement in the oxygenation outcome (P/F ratio) was modest and all 5 patients randomized to thrombolytic therapy died in the ICU. Considering the difficulty in recruitment and lack of a hint for benefit, these results dampen the enthusiasm for further testing of fibrinolytic therapy in critically ill patients with COVID-19-associaated ARDS.

Currently, there are no approved safe and efficient pharmacological therapies for COVID-19-associated ARDS. Among various pharmacological strategies, only corticosteroids have shown promising results vis-à-vis mortality and the need for invasive mechanical respiratory support (8). A high incidence rate of microthrombosis was one of the main reasons for several investigators to use escalated-dose anticoagulation in COVID-19, especially in critically ill patients, to improve gas exchange and hypoxemia. A small randomized controlled trial on 20 patients showed a statistically higher P/F ratio over 14 days in patients treated with therapeutic-dose compared with standard-dose anticoagulation, although their small sample size precluded a definitive recommendation (9). The INSPIRATION trial and 3 platform trials, namely REMAP-CAP, ACTIV-4, and ATTACC (1, 4), did not directly evaluate the P/F ratio as their primary outcome; nevertheless, their application of escalated-dose anticoagulation failed to translate into better survival, less need for circulatory support, or fewer thrombotic events.Higher-than-standard anticoagulation regimens have resulted in higher major bleeding events, prompting investigators to test other antithrombotic regimens (5). However, the primary application of

thrombolytic therapy in critically ill patients with COVID-19 has been confined to only a few case series. Wang et al in a case series of 3 patients with severe COVID-19-associated respiratory failure and ARDS reported that theoff-label intravenous administration of rtPA conferred an initial and at least a transientimprovement in oxygenation and the P/F ratio ranging from 38% to 100%. The improvement lasted in just1 patient, and the P/F ratio declined 48 and 33 hours after the completion of the rtPA infusion (2).The systemic administration of rtPA in previous studies on preclinical and human models of ARDS showed improvements in oxygenation (10). On the other hand, a multicenter cohort study on critically ill adults with COVID-19 recruited patients from the STOP-COVID registry, who received rtPA within 14 days after ICU admission for their suspected or confirmed pulmonary embolism or suspected pulmonary microthrombi, failed to show improvement in oxygenation or hemodynamics in the study patients. Additionally, major bleeding was reported in 10.2% of the patients within 7 days of the rtPA infusion. Importantly, administration of both therapeutic anticoagulation and tPA at the same time resulted in the increased risk of bleeding (in five out of six patients with major bleeding) (7).

We acknowledge several limitations; first, the study was not powered to formally prove or exclude the utility of fibrinolytic therapy in ICU patients with COVID-19 complicated by ARDS. The study design was open-label and each enrolling center and recruiting physicians were aware of group assignment. Also, our study population were severely ill with a median P/F ratio <100 and a median SOFA score >5 and markedly increased D-dimer (ng/mL) at baseline in all 3 study groups which might potentially impact the final results. None of the patients experienced major bleeding but due to the small sample size of the study the safety of the regimens cannot be concluded. Finally, since all patients assigned to rtPA, received therapeutic anticoagulation after

the termination of thrombolytic therapy, the potential confounding effect of latter treatment could not be ruled out.

Low-dose systemic thrombolytic therapy with rtPA did not appear to improve surrogate outcomes (P/F ratio and SOFA score) compared with standard-dose versus therapeutic-dose anticoagulation regimens. More evidence from larger clinical trials is needed to ascertain the efficacy and safety of rtPA in critically ill patients with COVID-19.

Disclosures: Dr. Bikdeli reports that he is a consulting expert, on behalf of the plaintiff, for litigation related to two brand models of IVC filters.

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Table 1. Baseline Characteristics of the Study Population			
Characteristics	**Treatment Assignments of the Study**		
	Standard-dose anticoagulation (n =5)	**Therapeutic-dose anticoagulation (n =5)**	**Tissue plasminogen activator (n =5)**
Age (y)	63 (48 – 65.5)	53 (43 – 67)	60 (55 – 64.5)
Gender (%) Female	2 (40.0)	0 (0	2 (40.0)
Current smoker	1 (20.0)	0 (0)	0 (0)
Diabetes mellitus	2 (40.0)	1 (20)	0 (0)
Hypertension	2 (40.0)	2 (40.0)	2 (40.0)
Hyperlipidemia	0 (0)	1 (20.0)	0 (0)
Ischemic heart disease	0 (0)	1 (20.0)	1 (20.0)
Chronic kidney disease	0 (0)	0 (0)	0 (0)
Malignancies	0 (0)	0 (0)	0 (0)
Obesity	1 (20.0)	1 (20.0)	1 (20.0)
History of pulmonaryembolism	0 (0)	0 (0)	1 (20.0)
History of deep veinthrombosis	0 (0)	1 (20.0)	0 (0)
Oral contraceptive pills	0 (0)	0 (0)	0 (0)
Aspirin	3 (60)	1 (20.0)	2 (40.0)
Captopril	0 (0)	1 (20.0)	0 (0)
Losartan	3 (60.0)	1 (20.0)	2 (40.0)
Atorvastatin	1 (20.0)	2 (40.0)	2 (40.0)

Hydroxychloroquine	4 (80)	1 (20)	5 (100)
Azithromycin	2 (40.0)	3 (60.0)	1 (20.0)
Naproxen	3 (60.0)	3 (60.0)	1 (25.0)
Dexamethasone	2 (40.0)	1 (20.0)	2 (50.0)
Methylprednisolone	3 (60.0)	3 (60.0)	3 (60.0)
Interferon-β	4 (80.0)	2 (40.0)	1 (20.0)
Hemoperfusion	3 (60.0)	1 (20.0)	1 (25.0)
Systolic blood pressure (mm Hg)	110 (107 – 130)	130 (120 – 140)	114 (105 – 127.5)
Respiratory rate (breaths per minute)	27 (17 – 29)	20 (15 – 25)	34 (18 – 39)
Heart rate (beats per minute)	92 (75 – 110.5)	91 (81.5 – 150)	103 (96.5 – 114.5)
Temperature (°C)	36.3 (36.15 – 37)	37.4 (36.75 – 37.8)	36.8 (36.65 – 37.25)
Duration of symptoms prior to hospitalization (d)	5 (4.5 – 6)	4 (3.5 – 7)	5 (3 – 6.5)
Duration of hospitalization prior to enrolment (d)	6 (4 – 9.5)	9 (6.5 – 11.5)	5 (1 – 9)
P/F ratio[2] at the time of randomization	46 (37 – 74.1)	62 (48.5 – 75.5)	55 (50.7 – 66)
Acute Physiology and Chronic Health Evaluation II score[3]	13 (5.5 – 16)	7 (4.5 – 11.5)	11 (4.5 – 14)
The Sequential Organ Failure Assessment score[4]	6 (5.5 – 7.5)	5 (4.5 – 9)	5 (4.5 – 7.5)

Fraction of inspired oxygen >50%, (%)	5 (100)	4 (100)	5 (100)
Vasopressor agent support within 72 h of enrollment (%)	1 (20.0)	1 (20.0)	1 (20.0)
Acute Respiratory Support at the Time of Randomization(%)			
Nasal cannula	0 (0)	0 (0)	0 (0)
Face mask	0 (0)	0 (0)	0 (0)
Reservoir mask	0 (0)	0 (0)	0 (0)
High-flow nasal cannula	0 (0)	0 (0)	0 (0)
Noninvasive positive pressure ventilation	2 (40.0)	2 (40.0)	1 (20.0)
Invasive positive pressure Ventilation (endotracheal intubation)	3 (60.0)	3 (60.0)	4 (80.0)
Peak expiratory flow (L/min)	31 (28 –.)	27 (26 –.)	35 (28 – 42.75)
Plateau pressure (cm H_2O)	28 (26 –.)	26 (26 – 26)	30 (25–.)
Positive end-expiratory pressure(cm H_2O)	9 (7–.)	10 (5–.)	10.5 (10 – 12.5)
Pulmonary compliance (mL/cm H_2O)	32 (23–.)	37.5 (33–.)	30 (28–.)
Fraction of inspired oxygen (FiO_2)	100 (100 – 100)	100 (96.25 – 100)	100 (97.5 – 100)
Peak inspiratory flow rate (L/min)	60 (60 – 60)	60 (60 – 60)	60 (60 –.)

Respiratory rate (breaths per minute)	25(17 – 30)	18 (18–.)	26.5 (24.5 – 29.25)
White blood cell count (cells/μL)	15300 (10900 – 18700)	7500 (5000 – 11850)	10450 (8425 – 10975)
Lymphocyte count (cells/μL)	610.1 (430.05 – 1105)	712.5 (712.5 – 712.5)	780 (523.2 –.)
Platelet count (cells/μL)	195000 (140500 – 226000)	188000 (110500 – 265500)	197000 (173500 – 274000)
Prothrombin time (s)	14 (1.6 – 16.6)	14.65 (4.47 – 27)	1.3 (1.11 – 8.45)
Activated partial thromboplastin time (s)	30 (27 – 33.5)	27.5 (23.[illegible] – 36)	33 (29 – 43)
Creatinine (mg/dL)	1 (0.8 – 1.15)	0.8 (0.7 – 0.85)	1 (0.8 – 1.05)
D-dimer (ng/mL)	15000 (7293 – 15000)	10000 (6340.5 – 10000)	10000 (7228.5 – 15000)

[1]All data are medians (Q1, Q3) unless stated otherwise.

[2]The Acute Physiology and Chronic Health Evaluation II (APACHE II)is an index for the evaluation of the illness severity based on the 3 components of the acute physiology score, age, and chronic health status with the score ranging from 0 to 71. Higher scores show poorer outcomes.

[3] The sequential organ failure assessment score (SOFA) is used to assess organ function status in patients admitted to the intensive care unit (ICU) according to 6 components:
the respiratory,cardiovascular,hepatic,coagulation,renal, and neurological systems.

[4] P/F ratio, Partial pressure of arterial oxygen (PaO_2) to the fraction of inspired oxygen (FiO_2) ratio. This index is a clinical indicator of hypoxemia and acute respiratory distress syndrome severity (ARDS severity: mild 200–300, moderate 100–200, and severe <100).

Table 2. Primary, Secondary, Exploratory, and Safety Outcomes			
Outcomes	**Standard-dose anticoagulation (n =5)**	**Therapeutic-dose anticoagulation (n =5)**	**Tissue plasminogen activator (n =5)**
Primary Outcome			
Median of percent change of P./F ratio within 48 hours of enrollment[1] (Q1, Q3)[2]	-15.8 (-28.0-24.1)	-7.6 (-14.5-68.9)	7.1 (-19.5-8.4)
Other Outcomes			
In-hospital mortality (%)	4 (80)	2 (40)	5 (100)
Median difference of the SOFA score within 48 hours of enrollment[3] (Q1, Q3)	1 (0 – 1)	0 (-1 – 0)	1 (0 – 2)
ICU discharge (%)	1 (20)	3 (60)	0 (0)
ICU stays (d)	11 (8 – 12)	8 (4 – 19)	7 (6 – 10)
Hospital stays (d)	11 (8 – 16)	16 (8 – 20)	7 (6 – 10)
Cerebrovascular accident (%)	0	0	0
Major bleeding[4] (%)	0	0	0
Severe thrombocytopenia[5] (%)	0 (0)	1 (20.0)	0 (0)

[1](Q1, Q3), interquartile range 25% - 75%

[2] P/F ratio, Partial pressure of arterial oxygen (PaO_2) to the fraction of inspired oxygen (FiO_2) ratio. This index is a clinical indicator of hypoxemia and acute respiratory distress syndrome severity (ARDS severity: mild 200–300, moderate 100–200, and severe <100).

[3] The sequential organ failure assessment score (SOFA) is used to assess organ function status in patients admitted to the intensive care unit (ICU) according to 6 components: the respiratory, cardiovascular, hepatic, coagulation, renal, and neurological systems.

[4] Major bleeding according to the International Society of Thrombosis and Haemostasis classification in nonsurgical patients

[5] Severe thrombocytopenia, defined as a platelet count <20000 (cell/μL)